

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S)	S. Denmeade, et al.	EXAMINER:	S. Liu
U.S.S.N.:	09/627,600	GROUP:	1653
FILED (U.S.):	July 28, 2000	Conf. No.	3631
FOR:	ACTIVATION OF PEPTIDE PRODRUGS BY (HK2)		

BOX SEQUENCE

Commissioner for Patents
Washington, D.C. 20231

Sir/Madam:

**RESPONSE TO NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT
APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID
SEQUENCE DISCLOSURES**

The following is in response to the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures, mailed July 3, 2002, in the above-referenced application.

Enclosed herewith for filing in the subject application are the following:

1. Paper Copy of Sequence Listing previously submitted;
2. New Computer Readable Form (CRF) of Sequence Listing;
3. Copy of Notice To Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acids Sequence Disclosures;
4. Copy of Error Report indicating unreadable disc;
5. Amendment directing entry of Paper Copy of Sequence Listing into specification [included in this paper];
6. Statement Accompanying Submission Of Sequence Listing Under 37 C.F.R. § 1.821(g) (included in this paper);
7. Associate Power of Attorney; and
8. Change of Correspondence Address.

Attorney Docket No. 57109 (71699)
U.S.S.N. 09/627,600
Filed (U.S.): July 28, 2000
Page 2 of 2

AMENDMENT DIRECTING ENTRY OF SEQUENCE LISTING

At the last page of the specification, please enter the attached paper copy of the Sequence Listing (25 pages) and number the pages as appropriate.

STATEMENT ACCOMPANYING SUBMISSION OF SEQUENCE LISTING

Provided herewith is a Paper Copy of a Sequence Listing for the nucleotide and/or amino acid sequence(s) in this application. Each Sequence has been assigned a separate identifier as required in 37 C.F.R. § 1.821(c) and 37 C.F.R. §§ 1.822 and 1.823. An amendment directing entry of the Paper Copy of the Sequence Listing into the specification is provided above.

Applicants further provide a Computer Readable Form (CRF) corresponding to the Paper Copy of the Sequence Listing provided herewith. Pursuant to 37 C.F.R. § 1.821(g), Applicants' agent hereby states that the CRF corresponds exactly to the Paper Copy of the Sequence Listing provided herewith. Applicants' agent further hereby states that the contents of the Paper Copy of the Sequence Listing and CRF do not go beyond the disclosure in the Application as filed and do not introduce new matter.

If for any reason, the fee paid is inadequate or credit is owed for any excess fee paid, you are hereby authorized and requested to charge Deposit Account No. **04-1105**.

Date: July 18, 2002

Respectfully submitted,

Dianne M. Rees
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Mailing Date: 7/18/02

Client: 71499

Inventors: Denmeade, et al

Serial No.: 09/427,400

Filing Date: 7/28/00

Attorney/Sec: Dym/Hunt

Docket No.: 57109

Patent No.:

Grant Date:

The dating stamp of the Patent and Trademark Office hereon will be taken as the date of filing of:

Response to Notice of Missing Requirements;
copy of paper sequence & disk;
copy of previously filed response 10/19/01
change of Address
associate Power of Attorney

Due Date: 8/3/02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Isaacs et al. Art Unit : 1653
Serial No. : 09/627,600 Examiner : H. Robinson
Filed : July 28, 2000
Title : ACTIVATION OF PEPTIDE PRODRUGS BY HK2

BOX SEQUENCE

Commissioner for Patents
Washington, D.C. 20231

RESPONSE TO NOTICE TO COMPLY WITH REQUIREMENTS
FOR PATENT APPLICATIONS CONTAINING
NUCLEOTIDE AND/OR AMINO ACID SEQUENCES

In response to the communication dated September 21, 2001 (copy enclosed), applicants submit herewith a Sequence Listing in computer readable form as required by 37 CFR §1.824. In addition, applicants submit an initial Sequence Listing as required under 37 CFR §1.823(a) and a statement under 37 CFR §1.821(f).

Applicants respectfully request entry of the paper copy and computer readable copy of the Sequence Listing filed herewith for the instant application. Furthermore, applicants request entry of the following amendments.

In the specification:

Insert the paper copy of the Sequence Listing filed herewith following the Oath/Declaration.

Replace the paragraph beginning at page 4, line 10, with the following rewritten paragraph:

--Fig. 1 is a portion of the amino acid sequence of Semenogelin I (SEQ ID NOs:1-4) and Semenogelin II (SEQ ID NOs:5-11), showing the cleavage sites for human kallikrein 2.--

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

October 19, 2001
Date of Deposit

Signature Shabreen Mehjabeen

SHAHREEN MEHTABEEN
Typed or Printed Name of Person Signing Certificate

Replace the paragraph beginning at page 5, line 29, with the following rewritten paragraph:

--Some examples of preferred peptides include (Note that the symbol][denotes an hK2 cleavage site):

1. Lys-Arg-Arg][(SEQ ID NO:12)
2. Ser-Arg-Arg][(SEQ ID NO:13)
3. Ala-Arg-Arg][(SEQ ID NO:14)
4. His-Arg-Arg][(SEQ ID NO:15)
5. Gln-Arg-Arg][(SEQ ID NO:16)
6. Ala-Phe-Arg][(SEQ ID NO:17)
7. Ala-Gln-Arg][(SEQ ID NO:18)
8. Ala-Lys-Arg][(SEQ ID NO:19)
9. Ala-Arg-Lys][(SEQ ID NO:20)
10. Ala-His-Arg][(SEQ ID NO:21)--

Replace the paragraph beginning at page 6, line 12, with the following rewritten paragraph:

--Additional preferred peptides of longer sequence length include:

11. Gln-Lys-Arg-Arg][(SEQ ID NO:22)
12. Lys-Ser-Arg-Arg][(SEQ ID NO:23)
13. Ala-Lys-Arg-Arg][(SEQ ID NO:24)
14. Lys-Lys-Arg-Arg][(SEQ ID NO:25)
15. His-Lys-Arg-Arg][(SEQ ID NO:26)
16. Lys-Ala-Phe-Arg][(SEQ ID NO:27)
17. Lys-Ala-Gln-Arg][(SEQ ID NO:28)
18. Lys-Ala-Lys-Arg][(SEQ ID NO:29)
19. Lys-Ala-Arg-Lys][(SEQ ID NO:30)
20. Lys-Ala-His-Arg][(SEQ ID NO:31)--

Replace the paragraph beginning at page 6, line 24, with the following rewritten paragraph:

--Additional preferred peptides that include an X-1 amino acid are:

21. Lys-Arg-Arg][Leu (SEQ ID NO:32)
22. Ser-Arg-Arg][Leu (SEQ ID NO:33)
23. Ala-Arg-Arg][Leu (SEQ ID NO:34)
24. Ala-Arg-Arg][Ser (SEQ ID NO:35)
25. His-Arg-Arg][Ala (SEQ ID NO:36)
26. Gln-Arg-Arg][Leu (SEQ ID NO:37)
27. Ala-Phe-Arg][Leu (SEQ ID NO:38)
28. Ala-Gln-Arg][Leu (SEQ ID NO:39)
29. Ala-Lys-Arg][Leu (SEQ ID NO:40)
30. Ala-Arg-Lys][Leu (SEQ ID NO:41)
31. Ala-His-Arg][Leu (SEQ ID NO:42)--

Replace the paragraph beginning at page 7, line 7, with the following rewritten paragraph:

--Preferred peptides of still longer sequence length having X₁ include:

32. His-Ala-Gln-Lys-Arg-Arg][Leu (SEQ ID NO:43)
33. Gly-Gly-Lys-Ser-Arg-Arg][Leu (SEQ ID NO:44)
34. His-Glu-Gln-Lys-Arg-Arg][Leu (SEQ ID NO:45)
35. His-Glu-Ala-Lys-Arg-Arg][Leu (SEQ ID NO:46)
36. Gly-Gly-Gln-Lys-Arg-Arg][Leu (SEQ ID NO:47)
37. His-Glu-Gln-Lys-Arg-Arg][Ala (SEQ ID NO:48)
38. Gly-Gly-Ala-Lys-Arg-Arg][Leu (SEQ ID NO:49)
39. His-Glu-Gln-Lys-Arg-Arg][Ser (SEQ ID NO:50)
40. Gly-Gly-Lys-Lys-Arg-Arg][Leu (SEQ ID NO:51)
41. Gly-Gly-His-Lys-Arg-Arg][Leu (SEQ ID NO:52)--

Replace the paragraph beginning at page 15, line 28, with the following rewritten paragraph:

--Recombinant hK2 was produced and purified as described in Lövgren et al., *Biochem. Biophys. Res. Co.*, 238, 549-555 (1997). Semenogelin I and II were isolated from human semen as described previously in Malm et al., *Eur. J. Biochem.*, 238, 48-53 (1996). The tripeptide aminomethylcoumarin (AMC) substrates Boc-Phe-Ser-Arg-AMC (SEQ ID NO:53), Boc-Gln-Gly-Arg-AMC (SEQ ID NO:54), H-Pro-Phe-Arg-AMC (SEQ ID NO:55), boc-Val-Pro-Arg-AMC (SEQ ID NO:56), H-D-Val-Leu-Lys-AMC (SEQ ID NO:57), Tos-Gly-Pro-Arg-AMC (SEQ ID NO:58), Tos-Gly-Pro-Lys-AMC (SEQ ID NO:59), Z-Leu-Leu-Arg-AMC (SEQ ID NO:60), Z-Val-Val-Arg-AMC (SEQ ID NO:61), Z-Ala-Arg-Arg-AMC (SEQ ID NO:62), and H-Arg-Gln-Arg-Arg-AMC (SEQ ID NO:63) were from Bachem (Bubendorf, Switzerland). The heptapeptide substrates Mu-Ala-Pro-Val-Leu-Ile-Leu-Ser-Arg-AMC (SEQ ID NO:64) and Mu-Val-Pro-Leu-Ile-Gln-Ser-Arg-AMC (SEQ ID NO:65) corresponding to the pro peptides of PSA hK2 were from Enzyme Systems Product (Livermore, CA, USA). ACT was purified from human blood plasma as described in Christensson et al., *Eur. J. Biochem.*, 194, 755-63 (1990). PCI was provided by Prof. Johan Stenflo (Malmö University Hospital, Malmö, Sweden), and SLPI, and PSTI by Prof. Kjell Ohlsson (Malmö University Hospital, Malmö, Sweden). Benzamidine hydrochloride was from Amresco® (Solon, OH, USA), leupeptin and antipain were from ICN Biomedicals (Costa Mesa, CA, USA), Aprotinin was from Sigma (St. Louis, MO, USA), and PPACK from Calbiochem (La Jolla, CA, USA).--

Replace the paragraph beginning at page 18, line 10, with the following rewritten paragraph:

--Substrates ending in either arginine or lysine were tested. The kinetic constants for hydrolysis of the substrates by hK2 are shown in Table 1. The best substrate was the kallikrein substrate Pro-Phe-Arg-AMC (SEQ ID NO:55) having the highest k_{cat} and k_{cat}/K_m values. The cathepsin B substrate Ala-Arg-Arg-AMC (SEQ ID NO:62) was also cleaved quite effectively having a relatively high k_{cat} value and a low K_m resulting in a four times lower k_{cat}/K_m value than that obtained for the kallikrein substrate Pro-Phe-Arg-AMC (SEQ ID NO:55). However, no

hydrolysis of Arg-Gln-Arg-Arg-AMC (SEQ ID NO:63) was detected. HK2 cleaved additionally Val-Pro-Arg-AMC (SEQ ID NO:56), and Leu-Leu-Arg-AMC (SEQ ID NO:60), but with lower efficiency. As with the semenogelins hK2 also here cleaves substrates with Arg at position P1 and preferentially a large residue or another Arg at position P2. None of the substrates with lysine in the C-terminal position were cleaved.--

Replace Table 1 beginning at page 19, line 1, with the following rewritten table:

--Table 1. Substrate Hydrolysis by hK2

Substrates	Km (M)	Kcat (min ⁻¹)	Kcat/km (μM ⁻¹ min ⁻¹)	Activity (%)
Pro Phe Arg-AMC (SEQ ID NO:55)	40	55	1.375	100
Val Pro Arg-AMC (SEQ ID NO:56)	48	1.6	0.034	6
Gly Pro Arg-AMC (SEQ ID NO:58)		NR		
Gly Pro Lys-AMC (SEQ ID NO:59)		NR		
Leu Leu Arg-AMC (SEQ ID NO:60)	71	2.4	0.034	7
Val Val Arg-AMC (SEQ ID NO:61)		NR		
Val Leu Lys-AMC (SEQ ID NO:57)		NR		
Phe Ser Arg-AMC (SEQ ID NO:53)		NR		
Gln Gly Arg-AMC (SEQ ID NO:54)		NR		
Ala Arg Arg-AMC (SEQ ID NO:62)	20	7.2	0.360	33
Arg Gln Arg Arg-AMC (SEQ ID NO:63)		NR		

Replace the paragraph beginning at page 19, line 3, with the following rewritten paragraph:

--The activity listed in Table 1 is the hydrolytic activity of hK2 with 100 μ M substrate in relation to the hydrolytic activity of hK2 with 100 μ M of the tissue kallikrein substrate H-Pro-Phe-Arg-AMC (SEQ ID NO:55). The entry "N.R." means that no reaction was detected.--

Replace the paragraph beginning at page 19, line 8, with the following rewritten paragraph:

--Activity of hK2 (1.6 pmol) was monitored using the substrate H-Pro-Phe-Arg-AMC (SEQ ID NO:55) (90 μ M). Inhibitors, at commonly used concentrations, and hK2 (8.3 nM) were mixed and proteolysis of 90 μ M H-Pro-Phe-Arg-AMC (SEQ ID NO:55) was followed up to 20 minutes, starting directly or 10 minutes after mixing the enzyme with various inhibitors. Inhibition was evaluated by comparison with enzyme-free controls.--

Replace the paragraph beginning at page 22, line 27, with the following rewritten paragraph:

--The progress of the reaction of hK2 (8nM final concentration) with the substrate Pro-Phe-Arg-AMC (SEQ ID NO:55) was monitored at two different substrate concentrations without or with different concentrations of PCI (80, 40 or 16 nM final concentration). The fluorescence measurements were started directly after mixing the enzyme with the inhibitor. The inhibition of hK2 by PCI could be described by the slow-binding inhibition mechanism presented in Scheme 2, which has been used in analyzing the interaction of PCI with various serine proteases (Hermans et al., *Biochem. J.*, 295, 239-245 (1993), and Hermans et al., *Biochemistry*, 33, 5440-44 (1994)). This mechanism assumes that a reversible complex is formed between the proteinase and serine proteinase inhibitor (serpin). The issues justifying the use of the slow binding inhibition mechanism despite the commonly held view that the serpin-proteinase complex is irreversible has been discussed in more detail by Hermans et al. (1993).--

Replace Table 4 beginning at page 25, line 1, with the following rewritten table:

--Table 4. Hydrolysis of hK2 Substrates

Peptide Sequence								hK2 Hydrolysis Rate (FU/hr/mg)	Serum Hydrolysis Rate FU/hr	
P7	P6	P5	P4	P3	P2	P1	P'1			
G	H	E	Q	K	R	R	L	(SEQ ID NO:66)	5966.31	0.17
G	G	G	K	A	R	R	L	(SEQ ID NO:67)	4784.22	0.03
G	G	G	K	A	H	R	L	(SEQ ID NO:68)	4100.94	0.09
G	P	A	H	Q	R	R	L	(SEQ ID NO:69)	4017.81	0.10
G	S	K	G	H	F	R	L	(SEQ ID NO:70)	3029.27	0.04
G	S	K	G	H	R	R	L	(SEQ ID NO:71)	2649.96	UD
G	K	D	V	S	R	R	L	(SEQ ID NO:72)	2316.12	0.08
G	S	Q	N	Q	R	R	L	(SEQ ID NO:73)	2100.48	0.05
G	S	Y	P	S	R	R	L	(SEQ ID NO:74)	2060.21	0.09
G	S	Y	P	S	S	R	L	(SEQ ID NO:75)	1456.18	0.06
G	H	E	Q	K	G	R	L	(SEQ ID NO:76)	650.80	0.04
G	S	N	T	E	R	R	L	(SEQ ID NO:77)	592.34	UD
G	S	Y	E	E	R	R	L	(SEQ ID NO:78)	324.75	0.04
G	K	D	V	S	G	R	L	(SEQ ID NO:79)	242.91	0.05
G	S	N	T	E	K	R	L	(SEQ ID NO:80)	255.90	0.13
G	S	K	G	H	F	H	L	(SEQ ID NO:81)	171.47	0.10
G	S	Q	N	Q	V	R	L	(SEQ ID NO:82)	193.55	0.03
G	P	L	I	L	S	R	L	(SEQ ID NO:83)	118.21	0.07
G	S	Y	E	E	R	H	L	(SEQ ID NO:84)	42.87	0.09
G	K	D	V	S	G	H	L	(SEQ ID NO:85)	67.55	0.05
G	G	G	K	A	H	H	L	(SEQ ID NO:86)	70.15	0.05
G	S	N	T	E	K	H	L	(SEQ ID NO:87)	80.54	0.03
G	P	A	H	Q	D	R	L	(SEQ ID NO:88)	75.34	0.06
G	H	E	Q	K	G	H	L	(SEQ ID NO:89)	1.30	UD
G	P	A	H	Q	D	H	L	(SEQ ID NO:90)	48.06	0.00
G	S	Y	P	S	S	H	L	(SEQ ID NO:91)	24.68	UD
G	S	Q	N	Q	V	H	L	(SEQ ID NO:92)	32.48	0.03--

Replace Table 5 beginning at page 26, line 1, with the following rewritten table:

--Table 5. Additional hK2 Substrates

Substrate Sequence								hK2 Hydrolysis Rate(FU/hr/mg)	Serum Hydrolysis Rate FU/hr	
P7	P6	P5	P4	P3	P2	P1	P'1			
G	H	A	Q	K	R	R	L	(SEQ ID NO:93)	3665.1	0.08
	G	G	K	S	R	R	L	(SEQ ID NO:94)	3439.7	0.03
G	H	E	Q	K	R	R	L	(SEQ ID NO:66)	3366.5	UD
G	H	E	A	K	R	R	L	(SEQ ID NO:95)	3324.1	UD
	G	G	Q	K	R	R	L	(SEQ ID NO:96)	3267.4	0.02
G	H	E	Q	K	R	R	A	(SEQ ID NO:97)	3051.5	0.06
	G	G	A	K	R	R	L	(SEQ ID NO:98)	2773.0	0.02
G	H	E	Q	K	R	R	S	(SEQ ID NO:99)	2638.5	UD
	G	G	K	K	R	R	L	(SEQ ID NO:100)	2583.0	UD
	G	G	H	K	R	R	L	(SEQ ID NO:101)	2428.4	UD
	G	G	K	A	F	R	L	(SEQ ID NO:102)	2374.2	0.07
G	A	E	Q	K	R	R	L	(SEQ ID NO:103)	2325.8	0.10
	G	G	K	A	Q	R	L	(SEQ ID NO:104)	2233.7	0.04
	G	G	K	A	R	R	L	(SEQ ID NO:105)	2171.2	UD
	G	G	K	Q	R	R	L	(SEQ ID NO:106)	2171.2	0.02
	G	G	K	H	R	R	L	(SEQ ID NO:107)	2079.2	UD
	G	H	E	Q	A	R	R	L	(SEQ ID NO:108)	1956.4
G	G	G	K	A	K	R	L	(SEQ ID NO:109)	1788.9	0.14
	H	E	Q	K	R	R	dL	(SEQ ID NO:110)	1690.9	0.15
	G	G	K	A	R	R	S	(SEQ ID NO:111)	1609.5	UD
	G	G	K	A	R	K	L	(SEQ ID NO:112)	1602.4	UD
G	H	E	Q	K	R	R	E	(SEQ ID NO:113)	1473.8	UD
	G	G	K	A	H	R	L	(SEQ ID NO:114)	1287.4	0.10
	G	G	K	A	N	R	L	(SEQ ID NO:115)	1113.9	0.01
	G	G	K	A	R	Q	L	(SEQ ID NO:116)	1021.9	0.13
	G	G	K	A	R	H	L	(SEQ ID NO:117)	939.3	UD
	G	G	K	A	R	N	L	(SEQ ID NO:118)	828.4	0.25
	G	G	K	A	dR	R	L	(SEQ ID NO:119)	494.4	0.06
	G	G	K	A	K	K	L	(SEQ ID NO:120)	77.9	UD
	G	G	K	A	H	K	L	(SEQ ID NO:121)	73.2	UD
	G	G	K	A	R	dR	L	(SEQ ID NO:122)	49.6	UD
	G	G	K	A	dR	dR	L	(SEQ ID NO:123)	16.5	UD--

In the claims:

Amend claims 8, 28, and 29 as follows:

--8. (Amended) The peptide of claim 6, wherein the amino acid sequence is selected from the group consisting of Ala-Gln-Lys-Arg-Arg (SEQ ID NO:124), Gly-Lys-Ser-Arg-Arg (SEQ ID NO:125), Glu-Gln-Lys-Arg-Arg (SEQ ID NO:126), Glu-Ala-Lys-Arg-Arg (SEQ ID NO:127), Gly-Gln-Lys-Arg-Arg (SEQ ID NO:128), Gly-Ala-Lys-Arg-Arg (SEQ ID NO:129), Gly-Lys-Lys-Arg-Arg (SEQ ID NO:130), Gly-His-Lys-Arg-Arg (SEQ ID NO:131), Gly-Lys-Ala-Phe-Arg (SEQ ID NO:132), Glu-Lys-Ala-Gln-Arg (SEQ ID NO:133), and Glu-Lys-Ala-Arg-Arg (SEQ ID NO:134).--

--28. (Amended) The composition of claim 17, wherein the peptide is Gly-Gly-Lys-Ala-Arg-Arg-Leu (SEQ ID NO:135).--

--29. (Amended) The composition of claim 17, wherein the therapeutic drug is a compound belonging to the group of thapsigargins which have been derivatized with a moiety containing a primary amine group, the peptide is Gly-Gly-Lys-Ala-Arg-Arg-Leu (SEQ ID NO:135), and the linker is selected from the group consisting of unsubstituted or alkyl-, aryl-, halo-, alkoxy-, alkenyl-, amido- or amino-substituted $\text{CO}-(\text{CH}=\text{CH})_{n1}-(\text{CH}_2)_{n2}-\text{Ar}-\text{NH}_2$, $\text{CO}-(\text{CH}_2)_{n2}-(\text{CH}=\text{CH})_{n1}-\text{Ar}-\text{NH}_2$, $\text{CO}-(\text{CH}_2)_{n2}-(\text{CH}=\text{CH})_{n1}-\text{CO}-\text{NH}-\text{Ar}-\text{NH}_2$, $\text{CO}-(\text{CH}=\text{CH})_{n1}-(\text{CH}_2)_{n2}-\text{CO}-\text{NH}-\text{Ar}-\text{NH}_2$, $\text{CO}-(\text{CH}_2)_{n3}-\text{NH}_2$, and $\text{CO}-(\text{CH}_2)_{n3}-\text{NH}-\text{CO}-\text{CH}(\text{R}_4)-\text{NH}_2$, wherein $n1$ and $n2$ are from 0 to 5, $n3$ is from 0 to 15, Ar is any substituted or unsubstituted aryl group, attachment of NH_2 to Ar is in a ortho, meta or para position with respect to the remainder of the linker, and R_4 is any naturally occurring amino acid side chain.--

Applicant : Isaacs et al.
Serial No. : 09/627,600
Filed : July 28, 2000
Page : 10

Attorney's Docket No.: 07265-191001 / DM-3575

REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. No new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: October 19, 2001

George E. Heibel
George E. Heibel, Ph.D.
Reg. No. 42,648

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"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 4, line 10, has been amended as follows:

Fig. 1 is a portion of the amino acid sequence of Semenogelin I (SEQ ID NOs:1-4) and Semenogelin II (SEQ ID NOs:5-11), showing the cleavage sites for human kallikrein 2.

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18. Lys-Ala-Lys-Arg][(SEQ ID NO:29)
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23. Ala-Arg-Arg][Leu (SEQ ID NO:34)
24. Ala-Arg-Arg][Ser (SEQ ID NO:35)
25. His-Arg-Arg][Ala (SEQ ID NO:36)
26. Gln-Arg-Arg][Leu (SEQ ID NO:37)
27. Ala-Phe-Arg][Leu (SEQ ID NO:38)
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29. Ala-Lys-Arg][Leu (SEQ ID NO:40)
30. Ala-Arg-Lys][Leu (SEQ ID NO:41)
31. Ala-His-Arg][Leu (SEQ ID NO:42)

Paragraph beginning at page 7, line 7, has been amended as follows:

Preferred peptides of still longer sequence length having X₁ include:

32. His-Ala-Gln-Lys-Arg-Arg][Leu (SEQ ID NO:43)
33. Gly-Gly-Lys-Ser-Arg-Arg][Leu (SEQ ID NO:44)
34. His-Glu-Gln-Lys-Arg-Arg][Leu (SEQ ID NO:45)
35. His-Glu-Ala-Lys-Arg-Arg][Leu (SEQ ID NO:46)
36. Gly-Gly-Gln-Lys-Arg-Arg][Leu (SEQ ID NO:47)
37. His-Glu-Gln-Lys-Arg-Arg][Ala (SEQ ID NO:48)
38. Gly-Gly-Ala-Lys-Arg-Arg][Leu (SEQ ID NO:49)
39. His-Glu-Gln-Lys-Arg-Arg][Ser (SEQ ID NO:50)
40. Gly-Gly-Lys-Lys-Arg-Arg][Leu (SEQ ID NO:51)
41. Gly-Gly-His-Lys-Arg-Arg][Leu (SEQ ID NO:52)

Paragraph beginning at page 15, line 28, has been amended as follows:

Recombinant hK2 was produced and purified as described in Lövgren et al., *Biochem. Bioph. Res. Co.*, 238, 549-555 (1997). Semenogelin I and II were isolated from human semen as described previously in Malm et al., *Eur. J. Biochem.*, 238, 48-53 (1996). The tripeptide aminomethylcoumarin (AMC) substrates [Bos] Boc-Phe-Ser-Arg-AMC (SEQ ID NO:53), Boc-Gln-Gly-Arg-AMC (SEQ ID NO:54), H-Pro-Phe-Arg-AMC (SEQ ID NO:55), boc-Val-Pro-Arg-AMC (SEQ ID NO:56), H-D-Val-Leu-Lys-AMC (SEQ ID NO:57), Tos-Gly-Pro-Arg-AMC (SEQ ID NO:58), Tos-Gly-Pro-Lys-AMC (SEQ ID NO:59), Z-Leu-Leu-Arg-AMC (SEQ ID NO:60), Z-Val-Val-Arg-AMC (SEQ ID NO:61), Z-Ala-Arg-Arg-AMC (SEQ ID NO:62), and H-Arg-Gln-Arg-Arg-AMC (SEQ ID NO:63) were from Bachem (Bubendorf, Switzerland). The heptapeptide substrates Mu-Ala-Pro-Val-Leu-Ile-Leu-Ser-Arg-AMC (SEQ ID NO:64) and Mu-Val-Pro-Leu-Ile-Gln-Ser-Arg-AMC (SEQ ID NO:65) corresponding to the pro peptides of PSA hK2 were from Enzyme Systems Product (Livermore, CA, USA). ACT was purified from human blood plasma as described in Christensson et al., *Eur. J. Biochem.*, 194, 755-63 (1990). PCI was provided by Prof. Johan Stenflo (Malmö University Hospital, Malmö, Sweden), and SLPI, and PSTI by Prof. Kjell Ohlsson (Malmö University Hospital, Malmö, Sweden). Benzamidine hydrochloride was from Amresco® (Solon, OH, USA), leupeptin and antipain were from ICN Biomedicals (Costa Mesa, CA, USA), Aprotinin was from Sigma (St. Louis, MO, USA), and PPACK from Calbiochem (La Jolla, CA, USA).

Paragraph beginning at page 18, line 10, has been amended as follows:

Substrates ending in either arginine or lysine were tested. The kinetic constants for hydrolysis of the substrates by hK2 are shown in Table 1. The best substrate was the kallikrein substrate Pro-Phe-Arg-AMC (SEQ ID NO:55) having the highest k_{cat} and k_{cat}/K_m values. The cathepsin B substrate Ala-Arg-Arg-AMC (SEQ ID NO:62) was also cleaved quite effectively having a relatively high k_{cat} value and a low K_m resulting in a four times lower k_{cat}/K_m value than that obtained for the kallikrein substrate Pro-Phe-Arg-AMC (SEQ ID NO:55). However, no hydrolysis of Arg-Gln-Arg-Arg-AMC (SEQ ID NO:63) was detected. HK2 cleaved additionally

Val-Pro-Arg-AMC (SEQ ID NO:56), and Leu-Leu-Arg-AMC (SEQ ID NO:60), but with lower efficiency. As with the semenogelins hK2 also here cleaves substrates with Arg at position P1 and preferentially a large residue or another Arg at position P2. None of the substrates with lysine in the C-terminal position were cleaved.

Table 1 beginning at page 19, line 1, has been amended as follows:

Table 1. Substrate Hydrolysis by hK2

Substrates	Km (M)	Kcat (min ⁻¹)	Kcat/km (μM ⁻¹ min ⁻¹)	Activity (%)
Pro Phe Arg-AMC (SEQ ID NO:55)	40	55	1.375	100
Val Pro Arg-AMC (SEQ ID NO:56)	48	1.6	0.034	6
Gly Pro Arg-AMC (SEQ ID NO:58)		NR		
Gly Pro Lys-AMC (SEQ ID NO:59)		NR		
Leu Leu Arg-AMC (SEQ ID NO:60)	71	2.4	0.034	7
Val Val Arg-AMC (SEQ ID NO:61)		NR		
Val Leu Lys-AMC (SEQ ID NO:57)		NR		
Phe Ser Arg-AMC (SEQ ID NO:53)		NR		
Gln Gly Arg-AMC (SEQ ID NO:54)		NR		
Ala Arg Arg-AMC (SEQ ID NO:62)	20	7.2	0.360	33
Arg Gln Arg Arg-AMC (SEQ ID NO:63)		NR		

Paragraph beginning at page 19, line 3, has been amended as follows:

The activity listed in Table 1 is the hydrolytic activity of hK2 with 100 μ M substrate in relation to the hydrolytic activity of hK2 with 100 μ M of the tissue kallikrein substrate H-Pro-Phe-[arg] Arg-AMC (SEQ ID NO:55). The entry "N.R." means that no reaction was detected.

Paragraph beginning at page 19, line 8, has been amended as follows:

Activity of hK2 (1.6 pmol) was monitored using the substrate H-Pro-[Lphe] Phe-[arg] Arg-AMC (SEQ ID NO:55) (90 μ M). Inhibitors, at commonly used concentrations, and hK2 (8.3 nM) were mixed and proteolysis of 90 μ M H-Pro-Phe-Arg-AMC (SEQ ID NO:55) was followed up to 20 minutes, starting directly or 10 minutes after mixing the enzyme with various inhibitors. Inhibition was evaluated by comparison with enzyme-free controls.

Paragraph beginning at page 22, line 27, has been amended as follows:

The progress of the reaction of hK2 (8nM final concentration) with the substrate Pro-Phe-Arg-AMC (SEQ ID NO:55) was monitored at two different substrate concentrations without or with different concentrations of PCI (80, 40 or 16 nM final concentration). The fluorescence measurements were started directly after mixing the enzyme with the inhibitor. The inhibition of hK2 by PCI could be described by the slow-binding inhibition mechanism presented in Scheme 2, which has been used in analyzing the interaction of PCI with various serine proteases (Hermans et al., *Biochem. J.*, 295, 239-245 (1993), and Hermans et al., *Biochemistry*, 33, 5440-44 (1994)). This mechanism assumes that a reversible complex is formed between the proteinase and serine proteinase inhibitor (serpin). The issues justifying the use of the slow binding inhibition mechanism despite the commonly held view that the seprin-proteinase complex is irreversible has been discussed in more detail by Hermans et al. (1993).

Table 4 beginning at page 25, line 1, has been amended as follows:

Table 4. Hydrolysis of hK2 Substrates

Peptide Sequence								hK2 Hydrolysis	Serum Hydrolysis	
P7	P6	P5	P4	P3	P2	P1	P'1	Rate (FU/hr/mg)	Rate FU/hr	
G	H	E	Q	K	R	R	L	(SEQ ID NO:66)	5966.31	0.17
G	G	G	K	A	R	R	L	(SEQ ID NO:67)	4784.22	0.03
G	G	G	K	A	H	R	L	(SEQ ID NO:68)	4100.94	0.09
G	P	A	H	Q	R	R	L	(SEQ ID NO:69)	4017.81	0.10
G	S	K	G	H	F	R	L	(SEQ ID NO:70)	3029.27	0.04
G	S	K	G	H	R	R	L	(SEQ ID NO:71)	2649.96	UD
G	K	D	V	S	R	R	L	(SEQ ID NO:72)	2316.12	0.08
G	S	Q	N	Q	R	R	L	(SEQ ID NO:73)	2100.48	0.05
G	S	Y	P	S	R	R	L	(SEQ ID NO:74)	2060.21	0.09
G	S	Y	P	S	S	R	L	(SEQ ID NO:75)	1456.18	0.06
G	H	E	Q	K	G	R	L	(SEQ ID NO:76)	650.80	0.04
G	S	N	T	E	R	R	L	(SEQ ID NO:77)	592.34	UD
G	S	Y	E	E	R	R	L	(SEQ ID NO:78)	324.75	0.04
G	K	D	V	S	G	R	L	(SEQ ID NO:79)	242.91	0.05
G	S	N	T	E	K	R	L	(SEQ ID NO:80)	255.90	0.13
G	S	K	G	H	F	H	L	(SEQ ID NO:81)	171.47	0.10
G	S	Q	N	Q	V	R	L	(SEQ ID NO:82)	193.55	0.03
G	P	L	I	L	S	R	L	(SEQ ID NO:83)	118.21	0.07
G	S	Y	E	E	R	H	L	(SEQ ID NO:84)	42.87	0.09
G	K	D	V	S	G	H	L	(SEQ ID NO:85)	67.55	0.05
G	G	G	K	A	H	H	L	(SEQ ID NO:86)	70.15	0.05
G	S	N	T	E	K	H	L	(SEQ ID NO:87)	80.54	0.03
G	P	A	H	Q	D	R	L	(SEQ ID NO:88)	75.34	0.06
G	H	E	Q	K	G	H	L	(SEQ ID NO:89)	1.30	UD
G	P	A	H	Q	D	H	L	(SEQ ID NO:90)	48.06	0.00
G	S	Y	P	S	S	H	L	(SEQ ID NO:91)	24.68	UD
G	S	Q	N	Q	V	H	L	(SEQ ID NO:92)	32.48	0.03

Table 5 beginning at page 26, line 1, has been amended as follows:

Table 5. Additional hK2 Substrates

Substrate Sequence								hK2 Hydrolysis Rate(FU/hr/mg)	Serum Hydrolysis Rate FU/hr	
P7	P6	P5	P4	P3	P2	P1	P'1			
G	H	A	Q	K	R	R	L	(SEQ ID NO:93)	3665.1	0.08
	G	G	K	S	R	R	L	(SEQ ID NO:94)	3439.7	0.03
G	H	E	Q	K	R	R	L	(SEQ ID NO:66)	3366.5	UD
G	H	E	A	K	R	R	L	(SEQ ID NO:95)	3324.1	UD
	G	G	Q	K	R	R	L	(SEQ ID NO:96)	3267.4	0.02
G	H	E	Q	K	R	R	A	(SEQ ID NO:97)	3051.5	0.06
	G	G	A	K	R	R	L	(SEQ ID NO:98)	2773.0	0.02
G	H	E	Q	K	R	R	S	(SEQ ID NO:99)	2638.5	UD
	G	G	K	K	R	R	L	(SEQ ID NO:100)	2583.0	UD
	G	G	H	K	R	R	L	(SEQ ID NO:101)	2428.4	UD
	G	G	K	A	F	R	L	(SEQ ID NO:102)	2374.2	0.07
G	A	E	Q	K	R	R	L	(SEQ ID NO:103)	2325.8	0.10
	G	G	K	A	Q	R	L	(SEQ ID NO:104)	2233.7	0.04
	G	G	K	A	R	R	L	(SEQ ID NO:105)	2171.2	UD
	G	G	K	Q	R	R	L	(SEQ ID NO:106)	2171.2	0.02
	G	G	K	H	R	R	L	(SEQ ID NO:107)	2079.2	UD
G	H	E	Q	A	R	R	L	(SEQ ID NO:108)	1956.4	0.14
	G	G	K	A	K	R	L	(SEQ ID NO:109)	1788.9	0.14
G	H	E	Q	K	R	R	dL	(SEQ ID NO:110)	1690.9	0.15
	G	G	K	A	R	R	S	(SEQ ID NO:111)	1609.5	UD
	G	G	K	A	R	K	L	(SEQ ID NO:112)	1602.4	UD
G	H	E	Q	K	R	R	E	(SEQ ID NO:113)	1473.8	UD
	G	G	K	A	H	R	L	(SEQ ID NO:114)	1287.4	0.10
	G	G	K	A	N	R	L	(SEQ ID NO:115)	1113.9	0.01
	G	G	K	A	R	Q	L	(SEQ ID NO:116)	1021.9	0.13
	G	G	K	A	R	H	L	(SEQ ID NO:117)	939.3	UD
	G	G	K	A	R	N	L	(SEQ ID NO:118)	828.4	0.25
	G	G	K	A	dR	R	L	(SEQ ID NO:119)	494.4	0.06
	G	G	K	A	K	K	L	(SEQ ID NO:120)	77.9	UD
	G	G	K	A	H	K	L	(SEQ ID NO:121)	73.2	UD
	G	G	K	A	R	dR	L	(SEQ ID NO:122)	49.6	UD
	G	G	K	A	dR	dR	L	(SEQ ID NO:123)	16.5	UD

In the claims:

Claims 8, 28, and 29 have been amended as follows:

8. (Amended) The peptide of claim 6, wherein the amino acid sequence is selected from the group consisting of Ala-Gln-Lys-Arg-Arg (SEQ ID NO:124), Gly-Lys-Ser-Arg-Arg (SEQ ID NO:125), Glu-Gln-Lys-Arg-Arg (SEQ ID NO:126), Glu-Ala-Lys-Arg-Arg (SEQ ID NO:127), Gly-Gln-Lys-Arg-Arg (SEQ ID NO:128), Gly-Ala-Lys-Arg-Arg (SEQ ID NO:129), Gly-Lys-Lys-Arg-Arg (SEQ ID NO:130), Gly-His-Lys-Arg-Arg (SEQ ID NO:131), Gly-Lys-Ala-Phe-Arg (SEQ ID NO:132), Glu-Lys-Ala-Gln-Arg (SEQ ID NO:133), and Glu-Lys-Ala-Arg-Arg (SEQ ID NO:134).

28. (Amended) The composition of claim 17, wherein the peptide is Gly-Gly-Lys-Ala-Arg-Arg-Leu (SEQ ID NO:135).

29. (Amended) The composition of claim 17, wherein the therapeutic drug is a compound belonging to the group of thapsigargins which have been derivatized with a moiety containing a primary amine group, the peptide is Gly-Gly-Lys-Ala-Arg-Arg-Leu (SEQ ID NO:135), and the linker is selected from the group consisting of unsubstituted or alkyl-, aryl-, halo-, alkoxy-, alkenyl-, amido- or amino-substituted $\text{CO}-(\text{CH}=\text{CH})_{n1}-(\text{CH}_2)_{n2}-\text{Ar}-\text{NH}_2$, $\text{CO}-(\text{CH}_2)_{n2}-(\text{CH}=\text{CH})_{n1}-\text{Ar}-\text{NH}_2$, $\text{CO}-(\text{CH}_2)_{n2}-(\text{CH}=\text{CH})_{n1}-\text{CO}-\text{NH}-\text{Ar}-\text{NH}_2$, $\text{CO}-(\text{CH}=\text{CH})_{n1}-(\text{CH}_2)_{n2}-\text{CO}-\text{NH}-\text{Ar}-\text{NH}_2$, $\text{CO}-(\text{CH}_2)_{n3}-\text{NH}_2$, and $\text{CO}-(\text{CH}_2)_{n3}-\text{NH}-\text{CO}-\text{CH}(\text{R}_4)-\text{NH}_2$, wherein $n1$ and $n2$ are from 0 to 5, $n3$ is from 0 to 15, Ar is any substituted or unsubstituted aryl group, attachment of NH_2 to Ar is in a ortho, meta or para position with respect to the remainder of the linker, and R_4 is any naturally occurring amino acid side chain.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Isaacs et al. Art Unit : 1653
Serial No. : 09/627,600 Examiner : H. Robinson
Filed : July 28, 2000
Title : ACTIVATION OF PEPTIDE PRODRUGS BY HK2

BOX SEQUENCE


Commissioner for Patents
Washington, D.C. 20231

VERIFIED STATEMENT UNDER 37 CFR §1.821(f)

I, Jennifer H. Payne, declare that I personally prepared the paper and the computer-readable copy of the Sequence Listing filed herewith for the above-identified application and that the content of both is the same.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of The United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: October 18, 2001


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Typed or Printed Name of Person Signing Certificate

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Denmeade, Samuel R.
Lilja, Hans

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<210> 57

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<213> Homo sapiens

<220>

<221> VARIANT

<222> 1

<223> Xaa = H-D (free amine without protecting group)

<221> VARIANT
 <222> 5
 <223> Xaa = AMC (aminomethylcoumarin)

<400> 57
 Xaa Val Leu Lys Xaa
 1 5

<210> 58
 <211> 5
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 <213> Homo sapiens

<220>
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 <222> 1
 <223> Xaa = Tos (4-Toluenesulphonyl)

<221> VARIANT
 <222> 5
 <223> Xaa = AMC (aminomethylcoumarin)

<400> 58
 Xaa Gly Pro Arg Xaa
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<210> 59
 <211> 5
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<220>
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 <222> 1
 <223> Xaa = Tos (4-Toluenesulphonyl)

<221> VARIANT
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 <223> Xaa = AMC (aminomethylcoumarin)

<400> 59
 Xaa Gly Pro Lys Xaa
 1 5

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 <223> Xaa = Z (carbobenzyloxy)

<221> VARIANT
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<223> Xaa = AMC (aminomethylcoumarin)

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Xaa Leu Leu Arg Xaa

1 5

<210> 61

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<221> VARIANT

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<223> Xaa = AMC (aminomethylcoumarin)

<400> 61

Xaa Val Val Arg Xaa

1 5

<210> 62

<211> 5

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<222> 1

<223> Xaa = Z (carbobenzyloxy)

<221> VARIANT

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<223> Xaa = AMC (aminomethylcoumarin)

<400> 62

Xaa Ala Arg Arg Xaa

1 5

<210> 63

<211> 6

<212> PRT

<213> Homo sapiens

<220>

<221> VARIANT

<222> 1

<223> Xaa = H (hydrogen)

<221> VARIANT

<222> 5

<223> Xaa = AMC (aminomethylcoumarin)

<400> 63

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<210> 64
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<213> Homo sapiens

<220>
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<223> Xaa = Mu (morphourea)

<221> VARIANT
<222> 10
<223> Xaa = AMC (aminomethylcoumarin)

<400> 64
Xaa Ala Pro Val Leu Ile Leu Ser Arg Xaa
1 5 10

<210> 65
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<213> Homo sapiens

<220>
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<223> Xaa = Mu (morphourea)

<221> VARIANT
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<210> 66
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<213> Homo sapiens

<400> 66
Gly His Glu Gln Lys Arg Arg Leu
1 5

<210> 67
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<400> 67
Gly Gly Gly Lys Ala Arg Arg Leu
1 5

<210> 68
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 Gly Gly Gly Lys Ala His Arg Leu
 1 5

<210> 69
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<400> 69
 Gly Pro Ala His Gln Arg Arg Leu
 1 5

<210> 70
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 1 5

<210> 72
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<210> 73
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<400> 73
 Gly Ser Gln Asn Gln Arg Arg Leu
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<210> 74
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<213> Homo sapiens

<400> 74

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1 5

<210> 75

<211> 8

<212> PRT

<213> Homo sapiens

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1 5

<210> 76

<211> 8

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<213> Homo sapiens

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Gly His Glu Gln Lys Gly Arg Leu
1 5

<210> 77

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<400> 77

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1 5

<210> 78

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<213> Homo sapiens

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Gly Ser Tyr Glu Glu Arg Arg Leu
1 5

<210> 79

<211> 8

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<210> 82
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<210> 83
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1 5

<210> 84
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1 5

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<210> 92
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<210> 93
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<400> 93

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<400> 95

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<210> 96

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<210> 97

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<210> 99

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<210> 108
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<400> 108
 Gly His Glu Gln Ala Arg Arg Leu
 1 5

<210> 109
 <211> 7
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 1 5

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 <223> Xaa = dL which is an isomer of Leu

<400> 110
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<400> 111
 Gly Gly Lys Ala Arg Arg Ser

1 5
 <210> 112
 <211> 7
 <212> PRT
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<400> 112
 Gly Gly Lys Ala Arg Lys Leu
 1 5

<210> 113
 <211> 8
 <212> PRT
 <213> Homo sapiens

<400> 113
 Gly His Glu Gln Lys Arg Arg Glu
 1 5

<210> 114
 <211> 7
 <212> PRT
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 Gly Gly Lys Ala His Arg Leu
 1 5

<210> 115
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 Gly Gly Lys Ala Asn Arg Leu
 1 5

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 Gly Gly Lys Ala Arg His Leu
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<210> 119
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 <213> Homo sapiens

<400> 120
 Gly Gly Lys Ala Lys Lys Leu
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<210> 121
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<400> 121
 Gly Gly Lys Ala His Lys Leu
 1 5

<210> 122
 <211> 7
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<220>
 <221> VARIANT
 <222> 6
 <223> Xaa = dR which is an isomer of Arg

<400> 122
 Gly Gly Lys Ala Arg Xaa Leu
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<210> 123
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<213> Homo sapiens

<220>

<221> VARIANT

<222> 5, 6

<223> Xaa = dR which is an isomer of Arg

<400> 123

Gly Gly Lys Ala Xaa Xaa Leu

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5

<210> 124

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<213> Homo sapiens

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Ala Gln Lys Arg Arg

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Gly Lys Ser Arg Arg

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5

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5

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Glu Ala Lys Arg Arg

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5

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<400> 128

Gly Gln Lys Arg Arg

1

5

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<400> 129
Gly Ala Lys Arg Arg
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Gly Lys Lys Arg Arg
1 5

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Gly His Lys Arg Arg
1 5

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Gly Lys Ala Phe Arg
1 5

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Glu Lys Ala Gln Arg
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Glu Lys Ala Arg Arg
1 5

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<400> 135

Gly Gly Lys Ala Arg Arg Leu
1 5

Express Mail Label No. EL 933049160 US
Docket No. 57109 (71699)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: S. Denmeade, et al.
U.S.S.N.: 09/627,600 Group: 1655
FILED: July 28, 2000 Confirmation No.: 3631
FOR: ACTIVATION OF PEPTIDE PRODRUGS BY (HK2)

Box Sequence
Commissioner for Patents
Washington, DC 20231

SIR:

LETTER

Applicants wish to bring to the attention of the Patent Office the following address
change for the Agent.

Agent's old correspondence address:

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45 Rockefeller Plaza
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New York, New York
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Facsimile: (212) 258-2291

Agent's new correspondence address:

EDWARDS & ANGELL, LLP
Dike, Bronstein, Roberts & Cushman
Intellectual Property Practice Group
P.O. Box 9169
Boston, Massachusetts 02209
Telephone: (617) 439-4444
Facsimile: (617) 439-4170

Applicants kindly ask that this address change for Agent be made of record in the above-identified application.

Please charge any fees that may be due in connection with this matter to Deposit Account 04-1105.

Please contact the undersigned attorney if any additional information is needed.

Respectfully submitted,



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Intellectual Property Group of
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P.O. Box 9169
Boston, MA 02209
(617) 439-4444

Customer No. 21874

Express Mail Label No. EL 933049160 US
Attorney Docket No. 57109 (71699)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S): S. Denmeade, et al.

U.S.S.N.: 09/627,600

FILED: July 28, 2000

FOR: ACTIVATION OF PEPTIDE PRODRUGS BY (HK2)

Box Sequence
Commissioner for Patents
Washington, D.C. 20231

.....
Sir:

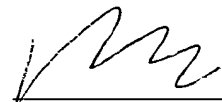
ASSOCIATE POWER OF ATTORNEY (37 CFR 1.34)

Please recognize the following as an Associate Agent in this case:

Dianne Rees, Reg. No. 45,281.

Respectfully submitted,

Date: 18 July 2002


Peter F. Corless (Reg. No. 33,860)
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